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BIO/CHEMICAL ANALYSIS OF DIOXIN-LIKE COMPOUNDS IN SEDIMENT SAMPLES FROM OSAKA BAY, JAPAN

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ABSTRACT

The combinatorial bio/chemical investigation of sediments (six surface samples and one core sample) from Osaka Bay, Japan was conducted to clarify the horizontal and vertical distribution profiles of persistent organic pollutants in the sediments. Concentrations of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs), and polybrominated diphenylethers (PBDEs) were determined by chemical analysis and compared to bioassay results using H4IIE-luc/Dioxin Responsive- Chemical Activated Luciferase eXpression (DR-CALUX). For surface sediments, World Health Organization-toxicity equivalent (WHO-TEQ) values ranged from 1.8 to 92 pg g⁻¹ dry weight and the bioassay-TEQ (CALUX-TEQ) values (3.7 – 140 pg g⁻¹ dry weight) yielded significant correlation with them ($r^2 = 0.96$). On the other hand, correlation between both TEQs (for WHO-TEQ, 5.5 – 47 and for CALUX-TEQ, 27 – 76 pg g⁻¹ dry weight) for core samples was not so good ($r^2 = 0.46$). Comparing the vertical profiles of CALUX-TEQ and WHO-TEQ, they were different in that WHO-TEQ reached the maximum in the 1957 core section, while CALUX-TEQ reached in the 1984 core section. CALUX-TEQ values were 1-5-fold more than WHO-TEQ values in all the surface and core samples. CALUX-TEQ values were calculated for PBDE and PBDD/F concentrations, employing their CALUX toxicity equivalent factors (CALUX-TEFs). The estimated CALUX-TEQ values obtained for the brominated compounds could explain for 11% on average (range 4.7 – 31%) of the experimentally obtained CALUX-TEQ values in the investigated surface sediments.

Keywords: CALUX, polyhalogenated dioxins (PCDD/Fs and PBDD/Fs), polybrominated diphenylethers (PBDEs), polychlorinated biphenyls (PCBs); sediment

INTRODUCTION

Recently, more focus has been put on sediment as an environmental media which may accumulate persistent organic pollutants (POPs) released into the aquatic environment. The content of POPs in sediment gives a significant indication of the general exposure to aquatic organisms and use of these compounds on land. Thus, it becomes quite important to profile toxic potency of POPs in sediments. Recent concern about POP contamination in sediment pressed the Government to set a new dioxin (polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and coplanar PCBs (Co-PCBs)) regulation value of 150 pg-WHO-TEQ (World Health Organization-toxicity equivalent) g⁻¹ dry weight for sediments in July, 2002, in Japan. Countermeasures (*e.g.*, dredging, sand covering and *in situ* solidification) for polluted sediments have been required to cut off a decisive route of dioxins to humans through the food chain, since we Japanese live on fish and more than 70% of our TEQ intake comes from fish consumption [1]. Also for PCBs, removal standard in sediment has been effectuated as

10 mg kg⁻¹ dry weight since 1975.

Besides PCDD/Fs and PCBs, increase in concentration of brominated compounds such as polybrominated dioxins (PBDD/Fs) and polybrominated diphenyl ethers (PBDEs) has been an emerging concern [2-4]. The brominated flame retardants (BFRs), PBDEs have been produced in large quantity and their concentrations in various environmental media are increasing rapidly, which may surpass the increase rate of PCBs which has been observed [5]. On the other hand, it is reported that thermal reactions of BFRs may form polybrominated-*p*-dioxins (PBDDs) and dibenzofurans (PBDFs) [6]. PBDD/Fs show similar effects such as aryl hydrocarbon receptor (AhR)-mediated activity (*e.g.*, ethoxyresorufin-*O*-deethylase activity) and toxicity (*e.g.*, wasting syndrome, thymic atrophy and liver toxicity in mammals) to PCDD/Fs [7].

In this study, surface and core sediments were collected from Osaka Bay which is surrounded by the most urbanized area in Japan. Their combinatorial bio/chemical investigation was conducted to clarify the horizontal and vertical POP distribution profiles of the sediments. PCDD/F, PCB,

PBDD/F and PBDE concentrations were analyzed using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). A different approach, a cell-based bioassay: DR-CALUX (Dioxin Responsive- Chemical Activated Luciferase gene expression) was applied to the sediment samples for a quantitative screening of dioxin-like POPs including the above substances. For the CALUX, the target compounds are ligands which bind to AhR in recombinant H4IIE-luc cells and the endpoint is based on AhR-mediated firefly (*Photinus pyralis*) luciferase gene expression [8]. Using the data from the chemical analysis, WHO-TEQ values were calculated and compared to CALUX-derived TEQ values (CALUX-TEQs) to account for contribution of PCDD/Fs and Co-PCBs to overall CALUX-TEQs. Additionally, by using the analytical concentrations of "CALUX-active" congeners of PBDEs and PBDD/Fs and their experimental CALUX-TEF (toxicity equivalent factor) values, their occupying contribution rate to CALUX-TEQs was estimated to survey the dioxin-like activity of the brominated compounds in sediments.

MATERIALS AND METHODS

Sediment Sampling

Sediment investigation was conducted using one core and six surface samples of Osaka Bay, whose locations were specified by using a Global Positioning System (Table 1 and Figure 1). The samples were taken in September, 2001. Surface samples were taken to a depth of two cm with a Smith and Macintyre bottom sampler and then mixed thoroughly, air-dried, crushed and stored in an airtight container. Core sample was taken vertically with acrylic pipe (length: 1.2 m, inside diameter: 10 cm) and subsampled into two-cm-thick pieces. Five subcores taken at the same point were sectioned and the corresponding layers were combined, dried in the same way as the surface samples. Sedimentation rates were determined by ^{210}Pb dating [9].

Analysis of PCDD/Fs, PCBs, PBDD/Fs and PBDEs

JIS K 0312 method [10] was basically adopted as clean-up method for HRGC/HRMS analysis of PCDD/Fs, PCBs, PBDD/Fs and PBDEs. Briefly, 25 g of dry sediment samples was Soxhlet-extracted with toluene for 16 h. Copper powder was added to a Soxhlet beaker to precipitate sulfur. One quarter of the crude extract (corresponds to 6.25 g) was spiked with $^{13}\text{C}_{12}$ -labeled PCDD/Fs and Co-PCBs and cleaned-up by eluting it through multilayer silica gel with 20% dichloromethane (DCM). Then the elution was concentrated and purified via an activated carbon dispersed silica gel column. Elution with hexane, DCM/hexane (1:3) and toluene yielded nonplanar PCBs in the first fraction, mono-ortho PCBs in the second fraction and non-ortho PCBs and PCDD/Fs in the third fraction. A quarter of the crude extract was spiked with $^{13}\text{C}_{12}$ -labeled PBDD/Fs and PBDEs and cleaned-up

through the multilayer silica gel and the following activated carbon dispersed silica gel column chromatography in the same way. The second and third fraction through the carbon column contained PBDEs and PBDD/Fs, respectively. The fractions were finally concentrated and processed to the HRGC/HRMS analysis for the quantification of objective congeners.

Bioassay Analysis Using CALUX

For the CALUX, 1/4 of the above described Soxhlet-extract (corresponds to 6.25 g) was used for clean-up. Copper treatment was also necessary during extraction for bioassay to remove sulfur which was cytotoxic. Column chromatography was conducted in a similar way as that for the chemical analysis without adding $^{13}\text{C}_{12}$ -labeled internal standards except that the elution through carbon column was conducted with DCM/hexane (1:3) and toluene. The first and second fractions were respectively evaporated, carefully reduced by nitrogen stream and replaced with small volume (20-50 μl) of dimethylsulfoxide (DMSO). Hereafter, the respective final DMSO fractions are called fraction 1 and fraction 2.

The CALUX was conducted using rat (H4IIE-luc) hepatoma cell line (provided by BioDetection Systems b.v., the Netherlands). This cell line was prepared as previously described by Aarts *et al.* [8]. The cells were plated in 96-well microplates in 100 μl of alpha-Minimal Essential Medium supplemented with 10% fetal bovine serum per well. The cells with at least 95% confluence 24 h after seeding were dosed in triplicate by adding 100 μl of the above medium with the assay extract to be tested dissolved in DMSO (0.4%). For each plate, TCDD standard series (0.3 – 300 pM) was included. After 24 h exposure, the dose medium was removed and the exposed wells were filled with 100 μl of phosphate buffer saline (PBS) containing 1 mM calcium and magnesium ions. PBS was renewed and 100 μl of LucLite assay substrate (Perkin-Elmer) was added to each well. The luciferase activity (light production) was measured in a luminometer (Atto) for 10 seconds per well.

After correction for background activity (DMSO solvent control), luciferase activities of diluted sample extracts causing response between that of 1 and 4 pM 2,3,7,8-TCDD were interpolated onto fitted TCDD calibration curve to express TCDD equivalent values (CALUX-TEQ) per gram dry sediment. Measurement was conducted in triplicate.

RESULTS AND DISCUSSION

Vertical and Horizontal POP Distribution

The first objective of this study was to determine concentrations of POPs (PCDD/Fs, PCBs, PBDD/Fs and PBDEs) in the surface and core sediment samples. The analytical results of sediment samples are summarized in Tables 2 and 3.

Table 1. Location description of sediment sampling points.

| Sediment | Surface 1 | Surface 2 | Surface 3 | Surface 4 | Surface 5 | Surface 6 | Core |
|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Sampling year | 2001 | 2001 | 2001 | 2001 | 2001 | 2001 | 2001 |
| Latitude | 34°40'42"N | 34°39'42"N | 34°38'42"N | 34°37'48"N | 34°36'18"N | 34°30'00"N | 34°38'04"N |
| Longitude | 135°24'24"E | 135°21'54"E | 135°25'12"E | 135°22'48"E | 135°26'12"E | 135°08'36"E | 135°20'00"E |
| Water depth (m) | 5 - 8 | 10 - 12 | 5 - 8 | 10 - 12 | 5 - 8 | approx. 36 | 13 - 15 |

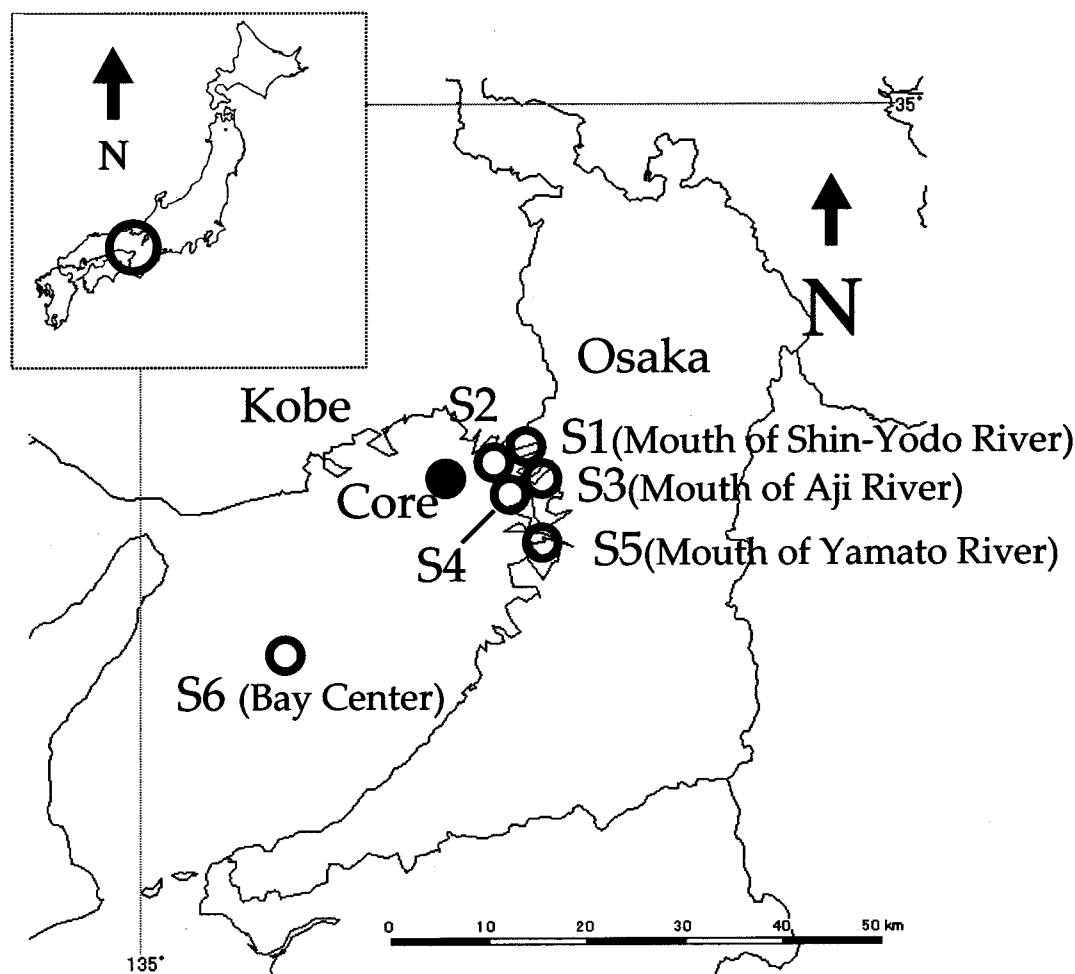


Figure 1. Map of the sampling points in Osaka Bay.

As for surface sediments (S1 – S5), PCDD/Fs and PCBs were detected at concentrations ranging from 5.2 to 12 and from 68 to 750 ng g⁻¹ dry weight, respectively, which showed these compounds were ubiquitous in the bay sediments surrounded by highly industrialized and urbanized area. S6 sediment showed quite lower values (0.5 ng g⁻¹ dry weight for PCDD/Fs and 8.9 ng g⁻¹ dry weight for PCBs) compared to other samples. The homologue profiles of PCDD/Fs resembled each other (data not shown). OCDD was predominant and HpCDDs were secondly higher contributors to PCDDs as previously observed in the Osaka and Tokyo Bay surface sediments in Japan [11, 12]. These two homologues accounted for 59 – 80% of the total PCDD/Fs in all the surface samples. PCB homologue patterns peaked at 3 - 6 chlorinated PCBs, which occupied 83 - 92% in the samples [13]. The concentrations of PCDD/Fs and Co-PCBs, expressed as the sum of WHO toxicity equivalents (WHO-TEQ) ranged from 1.8 to 92 pg g⁻¹ dry weight. PCDD/Fs contributed to more

than half of the total TEQ in all the surface samples.

For PBDD/Fs, TeBDDs, TeBDFs, PeBDFs, HxBDFs and HpBDFs were specifically quantified in the orders of pg g⁻¹ dry weight, showing similar trend as reported for Tokyo Bay sediments [14]. The concentration levels were at least one order of magnitude less than those of PCDD/Fs in every sample. The PBDE sum (MBDEs to DeBDE) was at the same level as the PCB sum with the exception of S6 (background level, not detected: < 6 ng g⁻¹ dry weight). As has been reported for Japanese sediments [11, 14], the most predominant congener was DeBDE (BDE-209) which were between 53 and 910 ng g⁻¹ dry weight. DeBDE concentrations tended to be higher in the mouth of rivers (S1, S3 and S5), but lower at offshore sights (S2, S4 and S6). TeBDEs, PeBDEs, HpBDEs, OBDEs and NBDEs were also detected in common in every river mouth samples, but at small concentrations up to ten ng g⁻¹ dry weight (data not shown).

As regards the results obtained for the core samples,

Table 2. Results of chemical- (PCDD/Fs and PCBs) and bioanalysis (CALUX) of sediment samples from Osaka Bay, in pg g⁻¹ dry weight.

| Sampling point | Designation | Chemical analysis | | | | | Bioanalysis | | | CALUX-TEQ/ WHO-TEQ |
|----------------|------------------------------------|-------------------|------------|---------|---------------------|-----------------|-------------------------|-------------------------|------------------|-----------------------|
| | | PCDD/ F sum | PCB sum | PCDD/Fs | WHO-TEQ, Co-PCBs | WHO-TEQ, sum | CALUX-TEQ, 1st frac. | CALUX-TEQ, 2nd frac. | CALUX-TEQ sum | |
| Surface 1 | Shin-Yodo River (river mouth) | 5,200 | 93,000 | 13 | 6.6 | 20 | 1.3 | 25.3 | 26.6 | 1.4 |
| Surface 2 | Shin-Yodo River (offshore) | 8,000 | 220,000 | 27 | 15 | 42 | 1.8 | 82.1 | 83.9 | 2.0 |
| Surface 3 | Aji River (river mouth) | 9,600 | 750,000 | 48 | 44 | 92 | 32.7 | 106.4 | 139.1 | 1.5 |
| Surface 4 | Aji River (offshore) | 5,900 | 82,000 | 13 | 5.8 | 19 | 0.8 | 24.1 | 24.9 | 1.3 |
| Surface 5 | Yanato River (river mouth) | 12,000 | 68,000 | 27 | 4.7 | 32 | 2.9 | 39.9 | 42.8 | 1.4 |
| Surface 6 | Center of Osaka Bay | 500 | 8,900 | 1.8 | 0.041 | 1.8 | 0.4 | 3.3 | 3.7 | 2.0 |
| Core 1 | Bay sediment core (mean date 2000) | 2,900 | 100,000 | 13 | 6.8 | 20 | 3.9 | 27.7 | 31.6 | 1.6 |
| Core 2 | Bay sediment core (mean date 1996) | 5,300 | 120,000 | 20 | 7.9 | 28 | 4.2 | 23 | 27.2 | 1.0 |
| Core 3 | Bay sediment core (mean date 1989) | 6,200 | 110,000 | 23 | 8.9 | 32 | 10.5 | 33.8 | 44.3 | 1.4 |
| Core 4 | Bay sediment core (mean date 1984) | 6,100 | 130,000 | 24 | 11 | 35 | 13.5 | 62.9 | 76.4 | 2.2 |
| Core 5 | Bay sediment core (mean date 1957) | 6,500 | 340,000 | 21 | 26 | 47 | 11.4 | 45.9 | 57.3 | 1.2 |
| Core 6 | Bay sediment core (mean date 1904) | 2,400 | 12,000 | 5.5 | 0.032 | 5.5 | 1.7 | 26.4 | 28.1 | 5.1 |

Table 3. Results of chemical analysis (PBDEs and PBDD/Fs) of sediment samples from Osaka Bay, in pg g⁻¹ dry weight.

| Sampling point | PBDEs | | PBDDs | | | | PBDFs | | | | | | PBDD/F sum |
|----------------|---------|----------|-------|-------|----------------|----------|-------|-------|-------|-------|------|----------|------------|
| | DeBDE | PBDE sum | TeBDD | PeBDD | Hx-, Hp-, OBDD | PBDD sum | PBDFs | | | | | | |
| | | | | | | | TeBDF | PeBDF | HxBDF | HpBDF | OBDF | PBDF sum | |
| Surface 1 | 900,000 | 910,000 | 3.6 | <0.1 | <0.2 | 3.6 | 35 | 44 | 120 | 140 | <0.2 | 340 | 340 |
| Surface 2 | 130,000 | 130,000 | 2.7 | <0.1 | <0.2 | 2.7 | 25 | 63 | 180 | 120 | <0.2 | 390 | 400 |
| Surface 3 | 560,000 | 590,000 | 3.7 | <0.1 | <0.2 | 3.7 | 45 | 38 | 87 | 130 | <0.2 | 290 | 300 |
| Surface 4 | 53,000 | 53,000 | 12 | 0.88 | <0.2 | 13 | 6.5 | 19 | 49 | 22 | <0.2 | 96 | 110 |
| Surface 5 | 120,000 | 130,000 | 20 | <0.1 | <0.2 | 20 | 63 | 120 | 280 | 100 | <0.2 | 570 | 590 |
| Surface 6 | <6,000 | <6,000 | 0.72 | <0.1 | <0.2 | 0.72 | 0.48 | <0.1 | <0.2 | 1.2 | <0.2 | 1.7 | 2.4 |
| Core 1 (2000) | 90,000 | 90,000 | 0.8 | <0.1 | <0.2 | 0.8 | 4.4 | 10 | 18 | 35 | <0.2 | 68 | 68 |
| Core 2 (1996) | 83,000 | 84,000 | 2.6 | 17 | <0.2 | 19 | 2.2 | 5.8 | 17 | 28 | <0.2 | 53 | 72 |
| Core 3 (1989) | 70,000 | 72,000 | 3.3 | <0.1 | <0.2 | 3.3 | 5.5 | 5.4 | 17 | 13 | <0.2 | 41 | 44 |
| Core 4 (1984) | 37,000 | 37,000 | 3.4 | <0.1 | <0.2 | 3.4 | 1.7 | 2.2 | 4.6 | 7.0 | <0.2 | 15 | 19 |
| Core 5 (1957) | <6,000 | <6,000 | 2.6 | <0.1 | <0.2 | 3.6 | 0.24 | <0.1 | <0.2 | <0.2 | <0.2 | 0.24 | 3.8 |
| Core 6 (1904) | <6,000 | <6,000 | 7.3 | <0.1 | <0.2 | 7.3 | <0.1 | <0.1 | <0.2 | <0.2 | <0.2 | <0.8 | 7.3 |

a high concentration of PCDD/F sum (6.5 ng g^{-1} dry weight) was detected from the section corresponding to 1957. PCDD/F concentrations were then leveled off and decreased after 1989. The increase of PCDD/Fs was due to the high level of OCDD and HpCDDs (data not shown) and the major contributor of these congeners could be pentachlorophenol (PCP) which contained them as impurities [15]. PCB showed a remarkable increase in the 1957 corresponding section (beginning of PCB production and use) and then decreased in the 1984 section and then leveled off until 2000, which was consistent with the trend of Co-PCBs observed in Tokyo Bay sediment core [16]. The PCB homologue pattern was dominated by 3 – 6 chlorinated PCBs, collectively accounting for 85 – 90% of PCB sum [13] as in the case of surface samples. WHO-TEQ peaked in the 1957 corresponding section (47 pg g^{-1} dry weight) and then gradually reduced until 2000 (20 pg g^{-1} dry weight). The contribution of PCDD/Fs to TEQ, which was dominant in the 1904 section, was reversed by Co-PCBs in the 1957 section. During 1957 and 1984, PCDD/F-TEQ became dominant again and then leveled off showing 70% of TEQ sum.

PBDEs were not detected in sections older than 1957 though their surface concentration of the core sample was in

the same order as that of PCBs, which shows a drastic increase of PBDEs after 1980s. DeBDE contributed to almost all the concentrations of PBDEs in the detected sections [17]. PBDD/Fs appeared in the 1904 section, but in a small concentration (7.3 pg g^{-1} dry weight). Their concentration increased in the 1984 slice and became around 70 pg g^{-1} dry weight in the top two sections. The vertical trends in PBDD/Fs seem similar to those in PBDEs. Although no proper explanation has been made for the potential sources of PBDD/Fs so far, different congener pattern compared to PCDD/Fs may be the key to elucidate them.

CALUX Activity by Sediment Extracts

As an example of the type of responses observed, the dose-response curves for the CALUX cells exposed to sediment extracts (fraction 1 and fraction 2) from the mouth of Yamato River (S5) were shown in Figure 2. The fraction 2 collected planar AhR ligands with high potency such as PCDD/Fs and non-ortho PCBs and clear elevation of luciferase induction was observed with the extract of 32 mg of S5 sediment, whereas the fraction 1 showed less induction potency compared to fraction 2, suggesting lower potency of

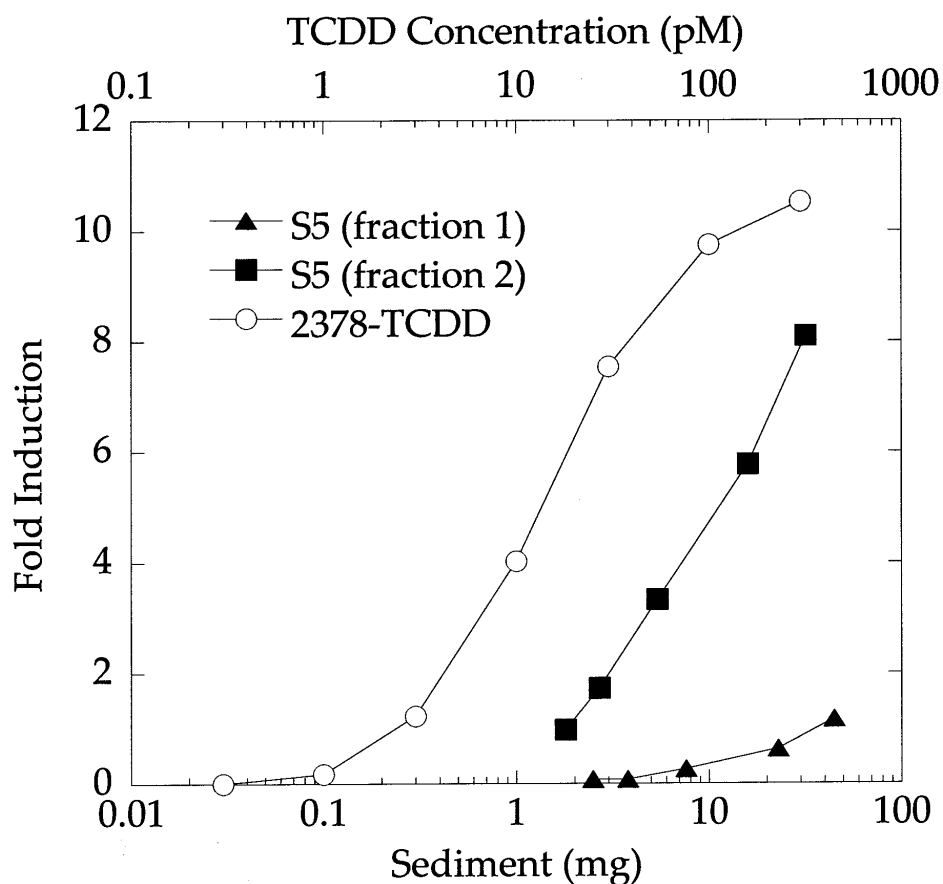


Figure 2. The CALUX response by extracts of surface sediment 5 (the mouth of Yamato River) and 2,3,7,8-TCDD.

slightly polar compounds such as mono-ortho PCBs and PBDEs contained in it. Similar trends were observed for all the tested sediment extracts. The linear part of the sample curves corresponding to 1 – 4 pM of TCDD calibration curve were focused on for the following quantitation of CALUX-TEQ.

CALUX-TEQs are summarized in Table 2 and Figure 3. CALUX analysis resulted in TEQ values for surface sediment samples (S1 – S6) with a range of 0.4 – 32.7 pg g⁻¹ dry sediment (fraction 1) and 3.3 – 106.4 pg g⁻¹ dry sediment (fraction 2), and core sediment samples (C1 – C6) with a range of 1.7 – 13.5 pg g⁻¹ dry sediment (fraction 1) and 23 – 62.9 pg g⁻¹ dry sediment (fraction 2).

The combinatorial analysis of the CALUX and HRGC/HRMS revealed excellent correlation between CALUX-TEQ (sum of fraction 1 and 2 results) and WHO-TEQ values for surface sediments. The relationship of both TEQs were quantified by the following regression equation: CALUX-TEQ (pg g⁻¹) = 1.6 WHO-TEQ (pg g⁻¹) - 0.1 (r² = 0.96). CALUX-TEQ value for fraction 2 contributed mostly to CALUX-TEQ sum. However, as an exception, for S3 sample, CALUX-TEQ showed highest value (139 pg g⁻¹) and fraction 1 (PCB-containing fraction) could account for 24% of the total value, which supports high contribution of Co-PCBs to total WHO-TEQ value as mentioned above. CALUX-TEQ/WHO-TEQ ratios were relatively consistent for surface sediments in the range of 1.3 – 2.0, which showed agreement with the bio-TEQ/chemical-TEQ ratios obtained for sediments using AhR-binding based bioassays [18, 19]. The presence of CALUX-activity over WHO-TEQ values supports the evidence for the existence of non-PCDD/F and non-PCB AhR agonists. Taking together the good correlation between CALUX-TEQ and WHO-TEQ and their ratios (more than 1-fold and less than 2-fold), it can be deduced that unknown dioxin-like compounds

occur and behave similarly to PCDD/Fs and Co-PCBs.

On the other hand, correlation between both TEQs for core samples was not so good (r² = 0.46) compared to surface sediment results. Comparing the vertical profiles of CALUX-TEQ and WHO-TEQ, they were different in that WHO-TEQ reached the maximum in 1957, while CALUX-TEQ reached it in 1984. The presence of significant dioxin-like activity (28 pg g⁻¹ dry sediment as the CALUX-TEQ) from the early 1900s (1904) was observed and this CALUX-TEQ value was derived from fraction 2 and was 5-fold greater than that of WHO-TEQ, indicating that the CALUX detected other unknown dioxin-like planar compounds. From the 1904 section to the 1984 section, the CALUX-TEQ in fraction 2 continued to increase. However, it is not clear whether composition of the causative active compounds in those core samples was consistent. Also it can be mentioned that for the core samples from the 1957 section downward, fraction 1 occupied 12 – 20% of the total CALUX-TEQ values, possibly reflecting the activity by Co-PCBs (*e.g.*, mono-ortho PCBs) included in PCBs of more than hundred ng g⁻¹ dry sediment.

Estimation Rate of PBDD/Fs and PBDEs to Overall CALUX-TEQs

Bioassay results (*i.e.*, CALUX and EROD) for sediment samples generally yields higher TEQ values than the chemical analysis. This is acceptable from the viewpoint to prevent false-negative evaluation in environmental monitoring. However, a bioassay-based approach will be further useful for toxicity identification and evaluation (TIE) when combined with instrumental analysis. Of all the identified and quantified PBDD/Fs and PBDEs in this study, four major congeners with AhR-binding potency were selected (Table 4).

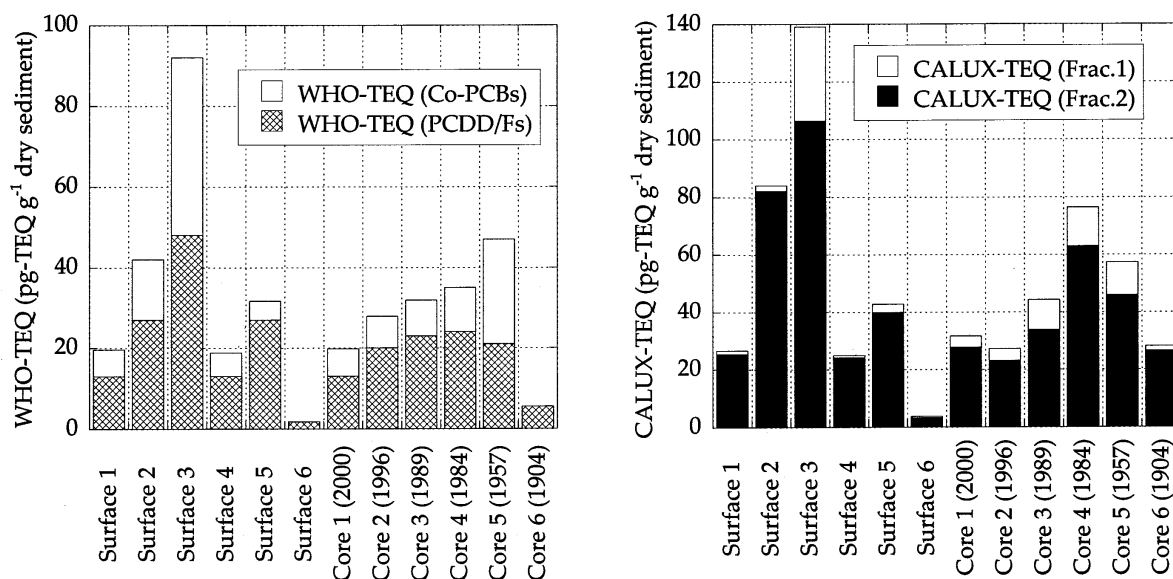


Figure 3. WHO-TEQ (left) and CALUX-TEQ (right) values in surface and core sediments from Osaka Bay.

Table 4. Concentrations of dioxin-like PBDE and PBDFs and calculated CALUX-TEQ derived from them, in pg g⁻¹ dry weight. CALUX-TEFs were expressed gravimetrically.

| Sampling point | Surface 1 | Surface 2 | Surface 3 | Surface 4 | Surface 5 | Surface 6 | Core 1 (2000) | Core 2 (1996) | Core 3 (1989) | Core 4 (1984) | Core 5 (1957) | Core 6 (1904) |
|--|-----------|-----------|-----------|-----------|-----------|-----------|------------------|------------------|------------------|------------------|------------------|------------------|
| Analytical concentrations | | | | | | | | | | | | |
| PBDE DeBDE (#209) | 900,000 | 130,000 | 560,000 | 53,000 | 120,000 | < 6,000 | 90,000 | 83,000 | 70,000 | 37,000 | < 6,000 | < 6,000 |
| PBDFs 2378-TeBDF | 5.0 | 11 | 7.8 | 1.2 | 3.7 | 0.48 | 3.4 | 2.2 | 1.4 | < 0.1 | 0.24 | < 0.1 |
| 12378-PeBDF | 1.0 | 3.3 | 1.4 | 0.96 | 3.8 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| 23478-PeBDF | 0.96 | 1.6 | 0.72 | 0.48 | 1.8 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| Calculated CALUX-TEQ derived from | | | | | | | | | | | | |
| DeBDE (CALUX-TEF: 5.4E-6) | 4.9 | 0.7 | 3.0 | 0.3 | 0.6 | | 0.5 | 0.4 | 0.4 | 0.2 | | |
| 2378-TeBDF (CALUX-TEF: 0.65) | 3.3 | 7.2 | 5.1 | 0.8 | 2.4 | 0.3 | 2.2 | 1.4 | 0.9 | | 0.2 | |
| 12378-PeBDF (CALUX-TEF: 0.075) | 0.1 | 0.2 | 0.1 | 0.1 | 0.3 | | | | | | | |
| 23478-PeBDF (CALUX-TEF: 0.069) | 0.1 | 0.1 | 0.05 | 0.03 | 0.1 | | | | | | | |
| Sum | 8.3 | 8.2 | 8.2 | 1.2 | 3.5 | 0.3 | 2.7 | 1.9 | 1.3 | 0.2 | 0.2 | 0 |
| CALUX-TEQ (experimental) | 26.6 | 83.9 | 139.1 | 24.9 | 42.8 | 3.7 | 31.6 | 27.2 | 44.3 | 76.4 | 57.3 | 28.1 |
| Calculated contribution rate of PBDE and PBDFs to CALUX- TEQ (%) | 31 | 9.8 | 5.9 | 4.7 | 8.1 | 8.4 | 8.5 | 6.9 | 2.9 | 0.3 | 0.3 | 0 |

DeBDE was selected from PBDEs because it was an overwhelmingly major congener and possessed higher CALUX-TEF (relative potency to TCDD based on EC₅₀) value of 5.4×10^{-6} (gravimetric based) out of 13 congeners tested so far [20]. 2,3,7,8-TeBDF, 1,2,3,7,8- and 2,3,4,7,8-PeBDFs were chosen from PBDD/Fs for the same reason (high occurrence and high CALUX-TEF values (based on EC₅₀)) with DeBDE, though they were four to five orders of magnitude less than DeBDE in concentrations, but four to five orders more potent in CALUX-TEF values [20]. By multiplying the analytical concentrations of the congeners by their experimental CALUX-TEFs, their theoretical CALUX-TEQs for the polybrominated compounds were calculated and their contribution rate to the experimentally obtained CALUX-TEQs was estimated. The calculation results showed the brominated compounds could explain for 11% on average (range 4.7 – 31%, 0.3 – 8.3 pg g⁻¹ dry weight as CALUX-TEQ) of the experimental CALUX-TEQ values in the investigated surface sediments. Especially, for S1 sediment, the calculated CALUX-TEQ values derived from the brominated compounds could complement fully the difference between CALUX-TEQ and WHO-TEQ. The major contributors to the calculated TEQ for the brominated compounds were DeBDE (8.5 – 59%) and 2,3,7,8-TeBDF (40 – 88%) for surface sediments.

For core samples, the calculated CALUX-TEQ values and their occupying rate increased from the 1984 section downward, resulting in 2.7 pg g⁻¹ dry weight (8.5% of the experimental CALUX-TEQ) in the 2000 section. By applying this kind of combination of bio/chemical analysis, the

contribution of the brominated compounds became clear to some extent. However, unexplained dioxin-like activity (CALUX-TEQ) was still remaining in the chemically stable fractions (*i.e.*, fractions 1 and 2 in this study). Refractory compounds such as other polyhalogenated aromatic hydrocarbons (PHAHs) are still in focus, taking some possible combined effects of such compounds and other factors into consideration.

CONCLUSIONS

Research results for sediment samples from Osaka Bay indicated that the CALUX were applicable for overall detection of mixtures of dioxin-like compounds. Throughout this study, CALUX-TEQ estimates became higher than WHO-TEQ values in all the investigated samples, nevertheless quite good correlation was observed between the CALUX- and WHO-TEQ values for surface sediments. Core sample analysis showed an interesting difference between vertical profiles of the CALUX- and WHO-TEQ, which gives us a two-dimensional (analytical/toxicological) history record of POP deposition. The existence of unknown active compounds has been basically suggested for the explanation of higher bioassay estimates. In order to answer such open questions, as well as contribution analysis of the newly known POPs such as PBDD/Fs and PBDEs dealt here, detailed chemical fractionation and identification of the active compounds will be important as the future work using the state-of-the-art fractionation and analytical/bioassay techniques.

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